QUANTITATIVE METHODS FOR ALLERGENIC FOOD RISK ASSESSMENT

Lutz Edler

Biostatistics Unit, German Cancer Research Center
Heidelberg, Germany

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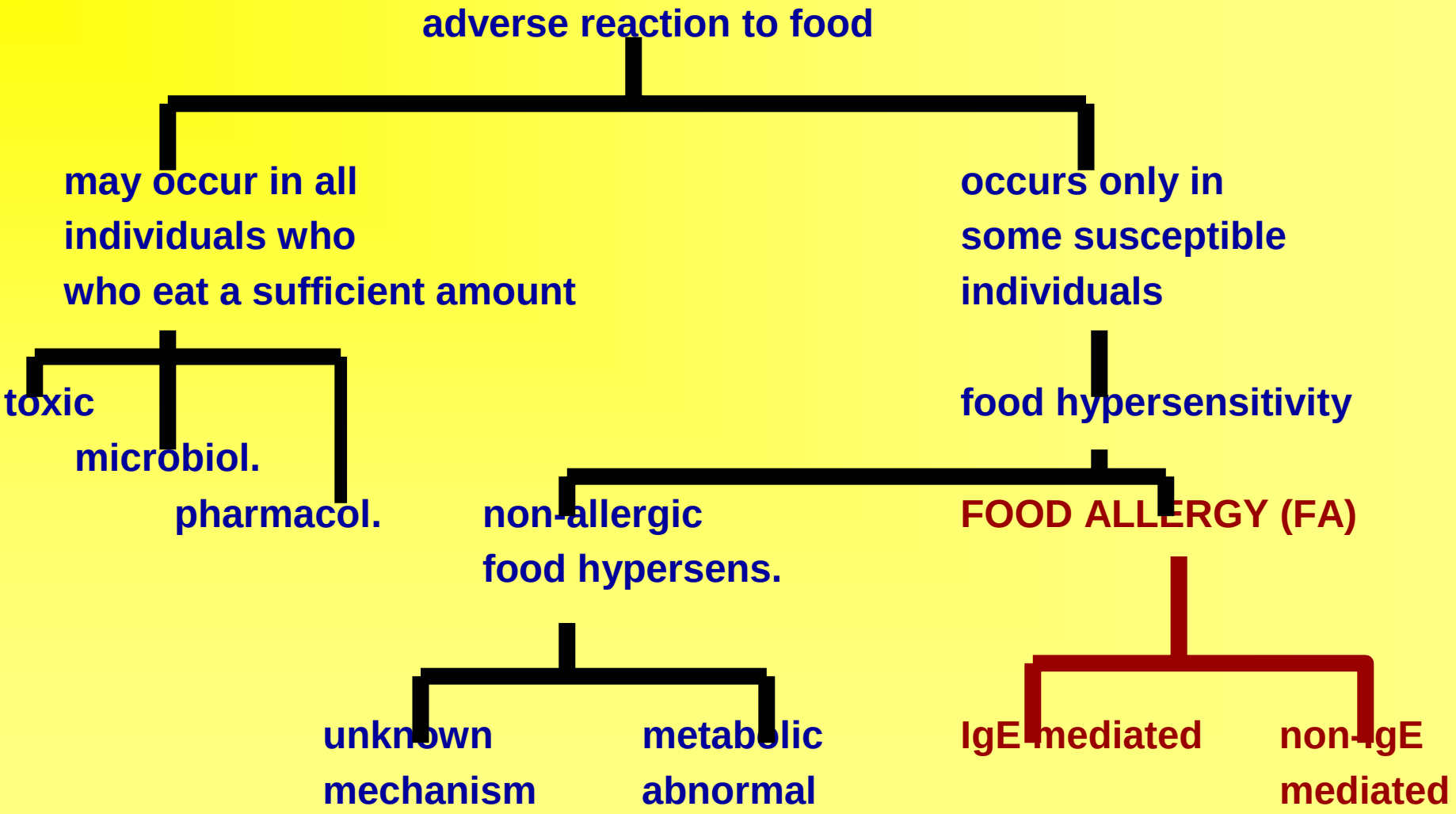
1.
Food Allergy

Food Allergy: Definition

- **Normal individuals develop a tolerance to ingested food proteins.**
- **Food Allergy is a hypersensitivity reaction initiated by immunologic mechanisms mediated by IgE antibodies or other immunological pathways.**

  Otherwise intolerance.
- **Food tolerance is broken down when IgE sensitization towards a food protein or a class of food proteins takes place**
  - **especially when the IgE immune system is still immature and physiologically incomplete as in infants**
- **Methods are of possible relevance for Genetically Modified Food (GMO)**

Food Allergy is one of several Adverse Reaction to Food



Mechanisms in Food Allergy: IgE mediated allergy

IgEs are antibodies, manufactured by B lymphocytes in response to foreign proteins (antigens or allergens)

IgE molecule has

- **an antigen specific end with affinity for the antigen (epitope)**
- **a receptor-specific end with affinity for the surface of immune cells(mast cells)**

Mast cells get sensitized to the allergen and subsequent re-exposure results in immediate IgE mediated allergic reaction.

The allergen triggers the cell to release mediators and to produce and release inflammatory substances which cause an inflammatory response

- **Overregulation and speed up of the immunological process is likely.**

Mast cells and related cells are in various parts of the body.

- **Food allergy can show both local and widespread generalized reactions.**
- **Non-allergic processes may mimic an IgE mediated allergic reaction by triggering mast cells to release mediators.**

Prevalence of Food Allergy

- **Prevalence of food allergy in the general population is estimated to about 2% in adults and about 8% in children.**
- **In the EU this amounts to about 8 million persons and about 3000 hospital admissions for which the primary diagnosis was anaphylactic shock (Crevel , 2001).**
- **Most prevalent is allergy to cow's milk constituents of about 2.2 - 5.2% (Ortolani et al, 2001)**
- **Prevalence of allergic diseases is increasing and an increase due to food is likely, in particular with the introduction of “new” foods.**
- **No specific drug treatment for food allergy has been established.**

Food Allergy: Response types

The response consists of two phases:

sensitization (induction)

elicitation (expression)

- with perhaps different doses necessary to give an effect
- with a high inter-individual variability in the elicitation phase

  peanut allergy: 100 μg -- 50 mg same effect were observed

Cross-effect type allergy:

Individuals with pollen allergy can also present allergy to food

Patients allergic to pollen produce IgE antibodies to food proteins that cross-react with respiratory allergens (of highly homologous protein structure).

epitope similarity

functional protein similarity

Food Allergy: Sensitization

Three forms of response are observed

- **the individual becomes tolerant not producing any immune response**
- **the individual develops an immune response involving cell-mediated immunity**
- **the individual becomes sensitized and develops an IgE mediated response with allergy**
  - personal or familial tendency (atopic)

- **Sensitization results from a complex interaction between the individual and the timing and nature of the first exposure to allergens.**
  - age dependency
  - in-utero sensitization

Expression of Food Allergy

Expression of Allergy

GI reactions
oral allergy syndrome (OAS)
acute vomiting and diarrhea
enterocolitic syndrome
eosinophilic gastroenteritis
gluten-sensitivity enteropathy

respiratory reactions
rhinitis
asthma
laryngeal edema

generalized reactions
anaphylaxis

cutaneous reactions
urticaria angioedema
atopic dermatitis
herpiform dermatitis

Sources:

- **milk**
- **nuts**
- **soy**
- **legumes (e.g., celery)**
- **fruit**
- **vegetables**

Food Allergy: Diagnosis

- **Asses the patient history**
- **Timely immediate relation**
- **Full dietary history**

- **Clinical test**
  - **skin test -** *local reaction is recorded*
    - **skin prick test (SPT)**
  - **blood test** - *search for specific antibodies*
    - **radioallergosorbent test (RAST),**
    - **fluorescent enzyme immunoassay (FEIA)**
  - **oral challenge in a clinical investigation**
    - **DBPCFC**
  - **response to dietary restrictions**
    - **elimination-reintroduction diets**

2.
Risk Assessment Task

Food Allergy: The Task

Determine the lowest dose of a food that can elicit a clinical reaction in the most sensitive patients.

LED lowest eliciting dose

"the regulator's wish"

MPD minimum provoking dose in a food challenge study

"clinical individuals's outcome"

level of food allergen

(log) amount of food

(log) protein

(log) allergen content / amount of allergen protein

Food Allergy: Annex IIIa of Directive 2003/89/EC

Food	MPD
Cereals	500 mg
Fish and crustaceans	mg / g for shrimps
Egg	µg – *low* mg
Peanut	µg
Soy	*low* µg
Milk	µg
Nuts	µg
Celery	mg
Mustard	µg
Sesame seed	mg
Sulphites (food additive)	20-50 mg

"reported dose", "may react at", "can be"

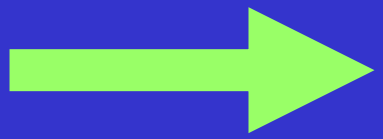
Food Allergen RA: The Issue

For the majority of the population there is no hazard and the risk even at extreme high doses is zero.

If a threshold for the elicitation of an allergic response can not be determined RA becomes extreme difficult for public health authorities and industry and it can not be avoided that more and more products are labeled as " may contain" the allergen.

This results in false positive warnings which do not help the consumer and may cause other health problems in the long run when more and more food items will carry this label.

3.
What Can Quantitative Risk Assessment Contribute?

general RA  allergen RA

Standard Quantitative Risk Assessment Advices

- estimate the risks associated with different levels of exposure
- determine health-based guidance values
  - ratio between the doses producing adverse effects and the current levels of human exposure/intake; ‘margin of exposure’
  - ratio between the NOAEL and the current levels of human exposure/intake; ‘margin of safety’
  - recommended minimum and maximum intake

- Those values will not be protective for individuals
  - who show extreme sensitivity, e.g. due to non-allergenic intolerance
  - who show allergenic reactions.

- Which advice should be given?
  - avoid exposure
  - adequate product labeling: The “may contain” problem.

Differences between allergenic RA and carcinogenic RA (Crevel,2001)

1. allergens are normal food constituents or normal environmental exposures, often making up a significant proportion of food and environmental exposure

2. genuine immune response with its two phases

3. minimum doses required to trigger a reaction in sensitive individuals

4. no well defined dose-response relationship

5. no accepted animal or *in vitro* model, no NOEAL, and no safety factors

6. cross-reactivity between food allergens and between food and inhalant allergens

Differences between allergenic RA and carcinogenic RA (Crevel,2001)

- prior knowledge on exposure
- prior knowledge on effect
- hazard identification
- hazard characterization

Prior knowledge on exposure

in general RA

- amount consumed per person
- pattern of exposure
- variation across population
- Animal Models available for a long time

in allergen RA

- could be too small for being determined
- occasional exposure plays an important role
- avoidance of consumption tendency
- Animal models not studied extensively, only recently
  - Brown Norway Rat Model and some others

Prior knowledge on effects

in general RA

- information on the substance and by-products, impurities and contaminants
- large spectrum of effects
- variation across populations
- sources of data
  - in vitro toxicity data
  - animal data
  - mechanistic studies
  - observational epidemiology
  - human studies

in allergen RA

- information on the substance
- small spectrum of effects in most cases
- dichotomous heterogeneity, large part of the population shows zero effect
- sources of data
  - *mechanistic studies*
  - *observational epidemiology*
  - human studies
    DBPCFC

Hazard Characterization

in general RA	in allergen RA
• Reference Dose (RfD) for lifelong exposure	• Acute Reference Dose (ARfD) for exposure over a period
• starting points of the (intake) dose: – NOAEL – LOEAL – BMD	• starting points of the (intake) dose need to be developed – NOAEL is not available study design ethical concerns
• external dose can be converted to human equivalent target organ dose using PBTK modeling	• PBTK modeling for immunological pathways is less developed

4.

Statistical Issues in a New Field of Quantitative Risk Assessment

i. What data are available?
ii. What methods are applied?
iii. What methods should be applied?
iv. What other designs should be discussed?

i. What data are available?

quantititative data
for dose-response

Double Blind Placebo Controlled Food Challenge

DBPCFC

Patients are challenged at increasing doses when symptom free under clinical control

During the challenge vital medical measurements are taken.

Occurrence of subjective and objective allergic symptoms are recorded and scored

An individual challenge is discontinued when objective symptoms occur or when subjective symptoms last for longer than 1 h.

An individual MINIMUM PROVOKING DOSE (MPD) is determined as the lowest dose eliciting a convincing allergic reaction.

Design and Analyis of the DBPCFC: Principle

Patients with a history of of adverse reactions and a positive Skin Prick Test or elevated IgE levels are selected **sub-population**

In/exclusion criteria are applied, baseline allergy related characteristics

Allergen is mixed into standardized challenge meal with dosed and placebo meal portions.

Increasing dose portions are randomly intersected with an equal number of placebos. **design and analysis of this information**

Patients and "feeders" are blinded.

Studies have been very small

Hourihane et al. (1997): n = 14
Wensing et al. (2002): n = 26

Design and Analyis of the DBPCFC: Example of doses

Dose group m	dose d_m (μg) peanut protein	
1.	30	
2.	100	
3.	300	
4.	1 000	= 1mg
5.	3 000	
6.	10 000	
7.	30 000	
8.	100 000	
9.	300 000	
10.	1 000 000	= 1g

2 separate challenges : doses 1. - 7. and doses 6. - 10.

4.

Statistical Issues

ii. What methods are applied?

Analyis of the DBPCFC: Endpoint

MPD = minimum provoking dose

The cumulative distribution function of MPD is estimated using

- a sample of size n
- curve fitting within a class of probability distributions.

The data are however more complex due to the design of the DBPCFC

- **directed sampling from low to high doses may cause a bias**
- **interval censored data**
- **intra-individual variability is not considered**

Design and Analyis of the DBPCFC

X = MPD = minimum provoking dose (mg allergen food protein per serving)

X random variable

$F(d) = P(X \leq d)$

X is considered as so-called individual "threshold dose" :

limit for that person for the occurrence of the effect

This is an old toxicological concept of risk assessment.

Tolerance Distribution Model

Any distribution function F(d) for a non-negative random variable can be used.

justification of the choice of F(d)

Analyis of the DBPCFC: Interval-Censored Data

MPD_i is not observed exactly but only an upper limit $UMPD_i$ is observed, i =1, ... , n.
$UMPD_i$ = 1 000 means $300 < MPD_i \leq 1000$.

95% CI are calculated according to Pearson-Clopper binomial method applied to the cumulative ratios **which is questionable**

Binomial model for $p(d) = P(MPD \leq d)$,

No intra-individual variability is estimated

- would need a repetition of the DBPCFC for that person

4.

Statistical Issues

ii. Which methods needed?

Analyis of the DBPCFC: Dose Response Model

In almost all applications one has used

$F(d) = P(X \leq d) = \Phi(d) = \Phi(d; \mu, \sigma)$ **Gaussian Normal**

Alternative

$F(d) = P(X \leq d) = 1 / \{1 + \exp(-[a+bd])\}$ **Logistic**

which allow for baseline values

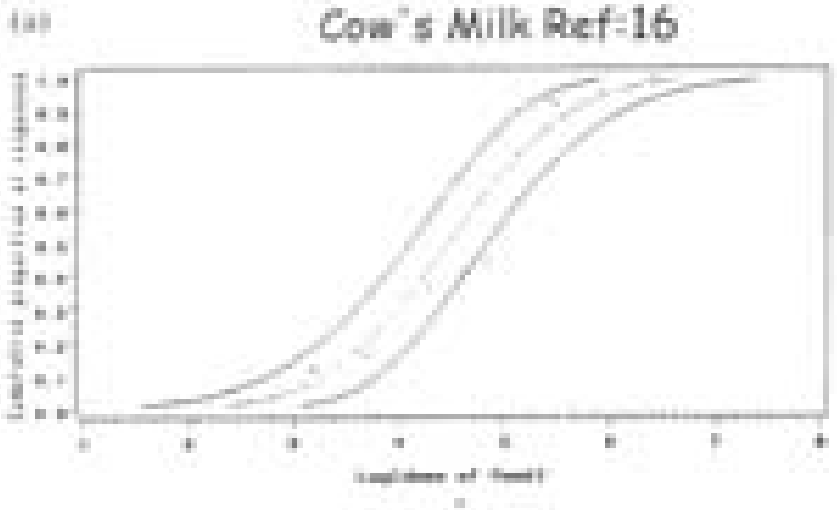
$F(d) = P(X \leq d) = 1 / \{1 + \exp(-[a+bd] + cx)\}$

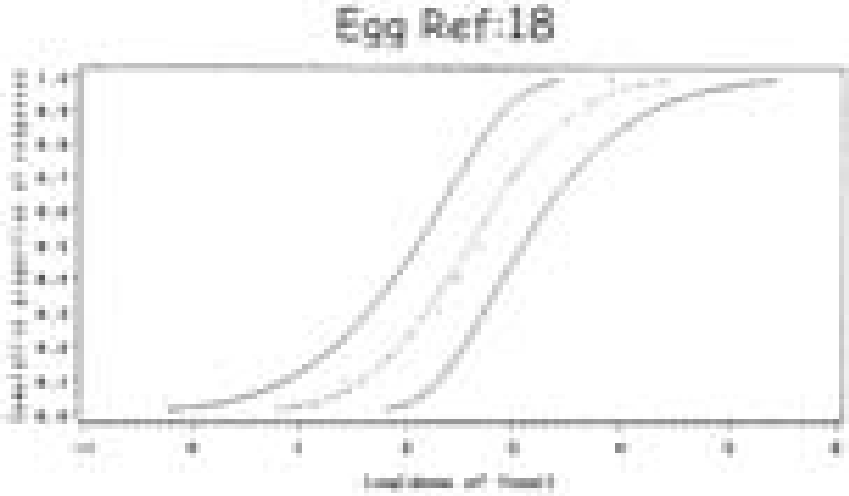
c: **gender, age,**
related symptoms,
SPT outcome, IgE, CAP outcome

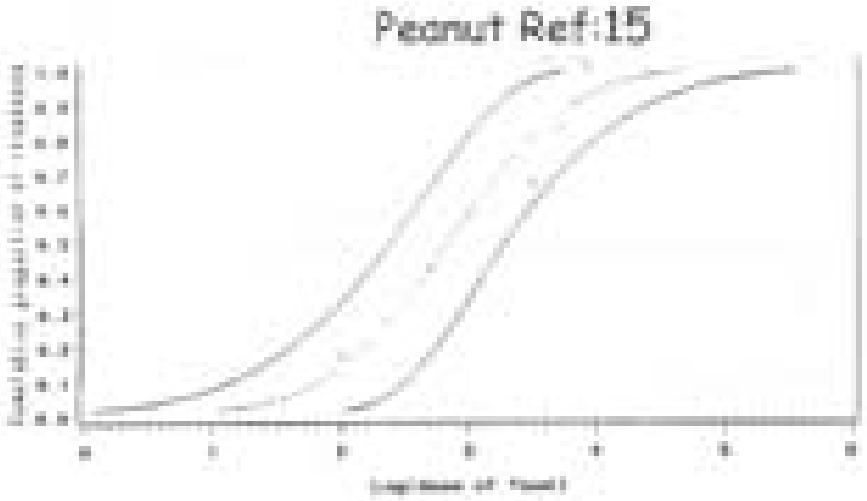
In applications d --> log(d)

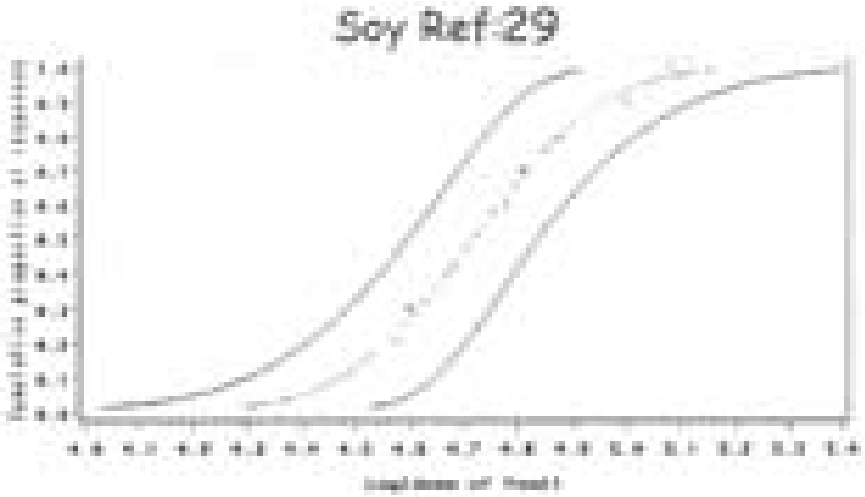
Fit of Cumulative Distribution

Blaabjerg-Jensen et al.









Fit of Cumulative Distribution

Hypothesis paper

Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature?

Background: The aim of this paper was to investigate whether a statistical model could be developed to estimate a "threshold" dose for foods eliciting allergic rejections in susceptible patients. The threshold dose is defined to be one that elicits allergic reactions in a given (small) proportion of susceptible patients, using data from published studies.

Methods: Based on data available from the literature, we developed a statistical model using the actual allergen content in the four foods, where data for allergen content are available (peanut, soy, egg, milk).

Results: The model demonstrated that the threshold doses giving a reaction of one in a million in susceptible patients were within the same order of magnitude for egg, milk and soy, but were an order of magnitude lower for peanut flour: 0.005 mg of cow's milk, 0.002 mg of fresh hen's egg, 0.0007 mg of peanut, or 0.0013 mg of soy flour.

Conclusions: Although several assumptions were made in creating this statistical model, we demonstrated that the previously published differences in threshold doses for various foods can be largely eliminated by comparing actual allergen content; this may therefore serve as a model for further studies.

C. Bindslev-Jensen[1], D. Briggs[2], M. Osterballe[1]

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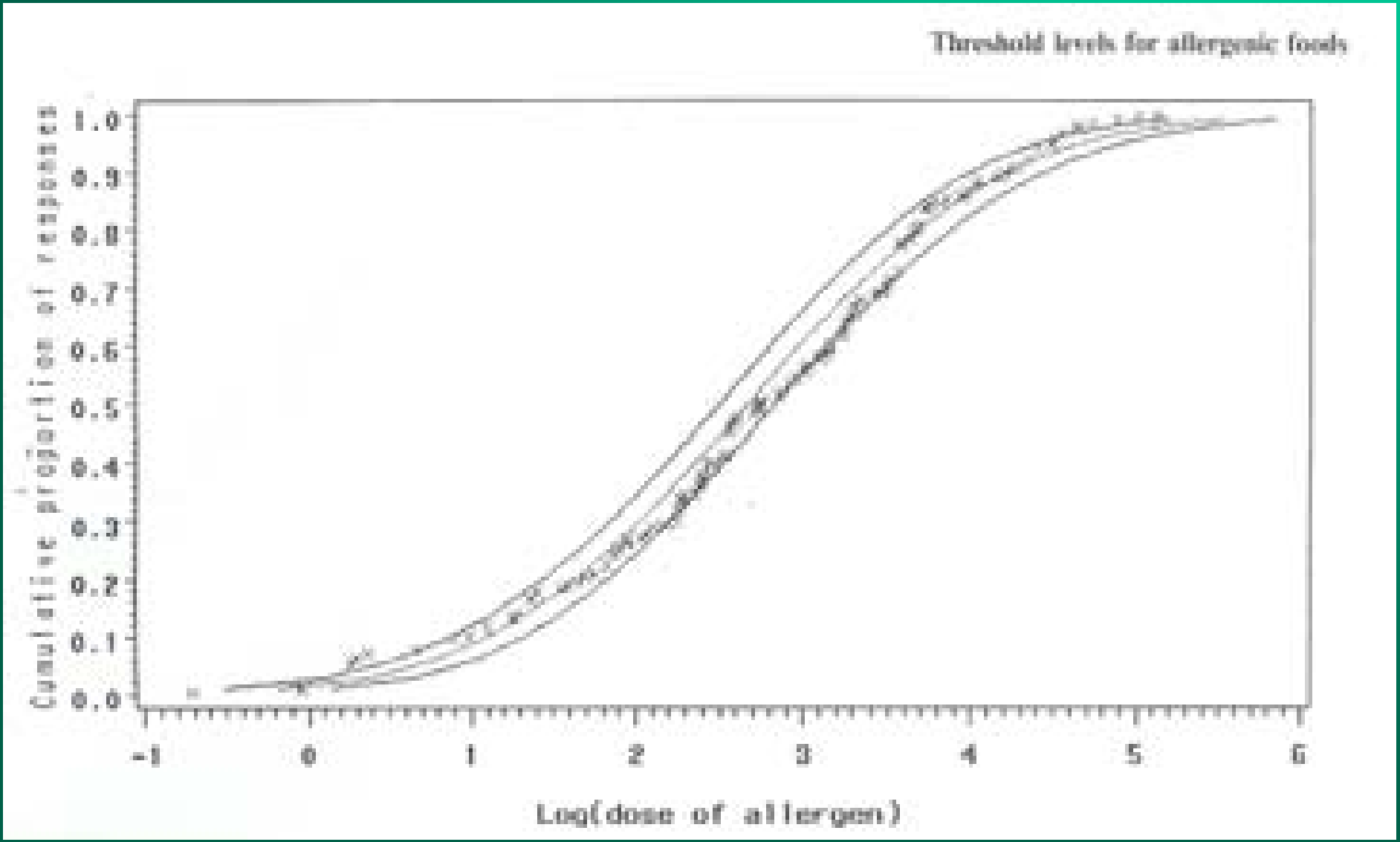
Fit of Cumulative Distribution



4.

Statistical Issues in a New Field of Quantitative Risk Assessment

ii. What other designs?

Analogy with Phase I Study

Exploit the analogy with pharmaceutical Phase I studies which aim at the determination of the maximum tolerated dose (MTD).

MTD is theoretically determined by an acceptable proportion θ of tolerable toxicity in the patient population. This proportion θ is in a cancer trial often set $\theta = 0.3$. The MTD is defined by

$$\theta = 0.3 = F(MTD) = P(X \leq MTD)$$

X random threshold dose of the patient

Generalized by individual grade dependent tolerance distribution:

X_g **= threshold dose for the occurrence of toxicity of grade g**

Stochastic ordering of $X_g : X_1 \geq X_2 \geq X_3 \geq X_4 \geq$ **X-death**

Analogy with Phase I Clinical Study

Phase I

- preselected set of increasing dose levels
- each patients is tested on one dose level only; dose-titration rarely used
- repeated test on one dose level with a small number of patients

Allergenic RA

- preselected set of increasing dose levels
- patients are tested on the same increasing set of dose levels; extended dose titration used
- no replicate data are obtained for one person

► What can be translated /transferred ?

Extension DBPCFC Design motivated by Phase I Designs

Examine the appropriateness of the dose space

- **tripling dose has been used in most applications**
- **vary the dose factor**
  - from 10 at very low doses to less than 2 for larger doses
  - use of Phase I modified Fibonacci scheme going down to 1.33
  - use individual Bayes design

Consider intra-individual variability

- only one ascending sequences is used at present
- re-challenge in the neighborhood of the MPD_i and determine the optimal estimate with s.e.

Extension DBPCFC Design motivated by Phase I Designs

Phase I designs adapted:

Traditional Escalation Rule (TER)

Up-and Down Rule (UDR)

Continual Reassessment Method (CRM) ------->

Full Bayesian Approach

BUT:

In contrast to Phase I trials more emphasis is put in allergy trials in the

intra-individual dose escalation (dose titration).

Extension of the Present DBPCFC Design: Adapt the CRM

Chose a family of dose response curves **F(d,a)**

Chose a prior density distribution for parameter a **g(a)**

Chose a target value a* such that

$$F(d^*, a^*) = \theta$$

θ denotes the target risk level, $\theta = 10^{-6} - 10^{-2}$

Given j-2 patients have been treated and the $j\text{-}1_{th}$ patient is under treatment up to the individual dose level no. k-1. Collect the dose response information as

$\{d_m\ y_{im}\ ; i = 1, \ldots, j\text{-}2, m = 1, \ldots, K_i\} = \Omega_{j\text{-}1k\text{-}1}$.

$\{d_{-1m}\ y_{j\text{-}1m}\ ;\quad m = 1, \ldots, k\text{-}1\}$

Consider for the next challenge the dose $d_{j\text{-}1k}$ and its outcome $y_{j\text{-}1k}$.

Chose the next dose $d_{j\text{-}1k}$ in an optimal Bayes way.

Extension of the Present DBPCFC Design: CRM

Calculate the posterior density of parameter a given this information

$$g(a; \Omega_{j-1,k}) = L(y_{j-1k}, d_{j-1k}, a)g(a, \Omega_{j-1k-1}) / \int L(y_{j-1k}, d_{j-1k}, u)g(u, \Omega_{j-1k-1})du$$

where

$$L(y_{j-1k}, d_{j-1k}, a) = F(d_{j-1k}, a)^{yj-1k} [1- F(d_{j-1k}, a)]^{1-yj-1k}$$

Calculate the current estimate of the target risk level $\theta = \theta_{i-1k}$ on the basis of Ω_{j-1k-1}

Determine d_{j-1k} such that θ_{i-1k} is next to the prefixed θ.

Extension of the Present DBPCFC Design: CRM

Chose a target value a* such that

$$F(d^*, a^*) = \theta$$

θ denotes the target risk level, $\theta = 10^{-6} - 10^{-2}$

This method will not work for low θ .

But it could work for moderate $\theta = 10^{-1}$ and would then provide a Benchmark type point estimate form which to start RA “as usual”

5.
Some Discussion Points

Discussion

WHICH POPULATION ?

Most sensitive patients are excluded form empirical studies.

Some studies include patients for which the lowest dose was positive and did not extend the FC to lower doses.

WHICH EXPOSURE ?

Food items may vary from origin and from production.

Food allergen content is calculated and must be considered as measurement with error.

Discussion

HOW TO USE THE PLACEBO INFORMATION ?

Present dose response analysis does not account for the in information obtained from the use of placebos.

What is the optimal design of using placebos?

at each dose level

randomize

HOW TO USE THE CONTROL POPULATION INFORMATION ?

Present designs do not use un-susceptible persons to estimate background response. **Ethical issues to consider!**

HOW TO USE THE MULTIVARIATE OUTCOME ?

symptom grading

Acknowledgment

ILSI EUROPE ***Eliciting Doses Expert Working Group***

David Briggs *Rene Crevel*

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Jonathan Hourihane *Andre Knulst*

Gilian Marmelstein *Josef Schlatter*

Finally

Madsen (2001):

In conclusion it is possible to use elements from chem
risk assessment in food allergy RA, but more knowledg
on the relationship between dose and response of diff
allergens in different patients populations is needed.

Patients **Hazardous Compound**

Allergenic Risk Assessment

Clinical Trials **Risk Assessment**

edler@dkfz.de

Back up Slides

Problem Formulation

HAZARD IDENTIFICATION

identification of adverse effects

- human studies
- animal -based toxicology
- in vitro toxicology
- structure-activity

HAZARD CHARACTERIZATION

- selection of critical data set
- mode/mechanism of action
- kinetic variability
- dynamic variability
- dose-response for critical effect
- identification for starting point for regulation

EXPOSURE ASSESSMENT

- levels of compound
  - food and diet
  - environment (air, water)
- amount of contact
- intake in individuals
- intake in special populations

Risk Characterization

Food Allergy: Allergenic Food

Cause-effect relationship is difficult to establish:

- **a single food can cause different symptoms in different individuals and at different times in one individual**
- **the same symptoms may be caused by different foods in the same and in different individuals**

- **ILSI categorization (1994/2003)**
  - **caused by food allergy** (e.g. anaphylaxis)
  - **associated with food allergy** (e.g. asthma)
  - **doubtful significance of food allergy** (e.g. migraine)
  - **caused by non-allergenic hypersensitivity** (e.g. lactose intolerance)

Food Allergy: Allergenic Food

Types of evidence for a food being termed allergenic

(Bousquet et al. (1998)

- **positive result in a DBPCFC = Double Blind Placebo-Controlled Food Challenge clinical study**
- **detailed reporting of a fatal or life-threatening anaphylactic reaction where food is clearly implicated**

<u>THE BIG EIGHT</u>:

Wheat

Crustaceans

Eggs

Fish

Peannut, soybean

Milk

Tree nuts

Sesame seed

Food Allergy: Two Perspectives

The patient perspective:

Avoidance of all relevant food

But: cross-contact is possible

cross-reactivity is possible

The industry perspective:

Clean one-purpose production with detailed list of ingredients

But: multi-purpose production

trace carry-over from production

introduced through goods of other manufacturers

Allergenic RA of Food from Genetically Modified Crop Plants
FAO/WHO Decision Tree from 2000

Is the source of the gene allergenic ? :

NO

Is there sequence similarity between the GMO and known allergenes?

N: Is the GMO stable to digestions and processing?

N: NO EVIDENCE OF ALLERGENICITY Y: POSSIBLY ALLERGENIC

__Y: PERFORM Solid Phase Immunoassay differentially: ***

YES: PERFORM Solid Phase Immunoassay :

Is the solid phase immunoassay positive? Y: ALLERGENIC

N: Is the skin brick test positive ? Y: ALLERGENIC

N: Is the DBPCFC positive?

N: NON-ALLERGENIC Y: ALLERGENIC

Allergenic RA of Food form Genetically Modified Crop Plants
FAO/WHO Decision Tree from 2000

Y: PERFORM Solid Phase Immunoassay differentially: *

YES: continue as above

NO: Is the source is from a commonly allergenic source?

Y: Continue as above

N: Less than 5 individual samples negatively tested?

GOTO Stability test

N: More than 5 individual samples negatively tested?

NO EVIDENCE OF ALLERGENICITY

Allergenic RA of Food Derived from Biotechnology
FAO/WHO Decision Tree from 2001

Is the source of the gene allergenic ? :

NO

Is there sequence homology with known allergenes?

N: Targeted serum screen positive?

N: Pepsin restistance test / animal model

+/+ +/- -/-

high medium low PROBABILITY OF ALLERGENICITY

Y: LIKELY ALLERGENIC

YES: Is there sequence homology with known allergenes?

N: Specific serum screen positive?

N:Targeted serum screen positive? as above

Y: LIKELY ALLERGENIC

Food Allergy: The Risk Assessment Need

The food labeling problem:

- lengthy list for a large number of ingredients
- 25% rule and 5% rule is not applicable

defensive policy:

- EU Directive list of allergen labeling
- “guaranteed xxx free” must not be 0.0 in practice

offensive policy:

- positive declaration labeling
- “may contain traces of xxx”

Dose-response Information from the DBPCFC Factors of influence:

- **clinical patient selection**
  - very sensitive patients not challenged due to risks of severe reactions
  - in-vitro and in vivo test used for the selection of patients
- **nature of suspected reaction**
- **source of allergen (food)**
- **starting dose of challenge (sub-clinical reaction)**
- **dose increment (fold rules)**
- **time interval (15-60 min, 48 h)**
- **top dose (range of normal intake)**
- **number of challenges (one verum and one placebo)**
- **statistical evaluation**
  - individual evaluation
  - Should patients reacting to placebo be excluded?
  - group evaluation

Design and Analyis of the DBPCFC: Wensing et al. (2002): n = 31

Dose group m	dose d_m (μg) hazelnut protein	
4.	1 000	= 1mg
5.	3 000	3
6.	10 000	10
7.	30 000	30
8.	100 000	100
9.	300 000	300
10.	1 000 000	= 1g

Use of Bootstrap sampling for curve estimation should be considered.

Food Allergy: DBPCFC oral challenge

DBPCFC = Double Blind Placebo Controlled Food Challenge

Difficulties in blinding

Difficulties in dose calculation

Manufacturing quality of placebo and treatment capsules

Not applicable for patients with lifethreatening history

Reponse evaluation:

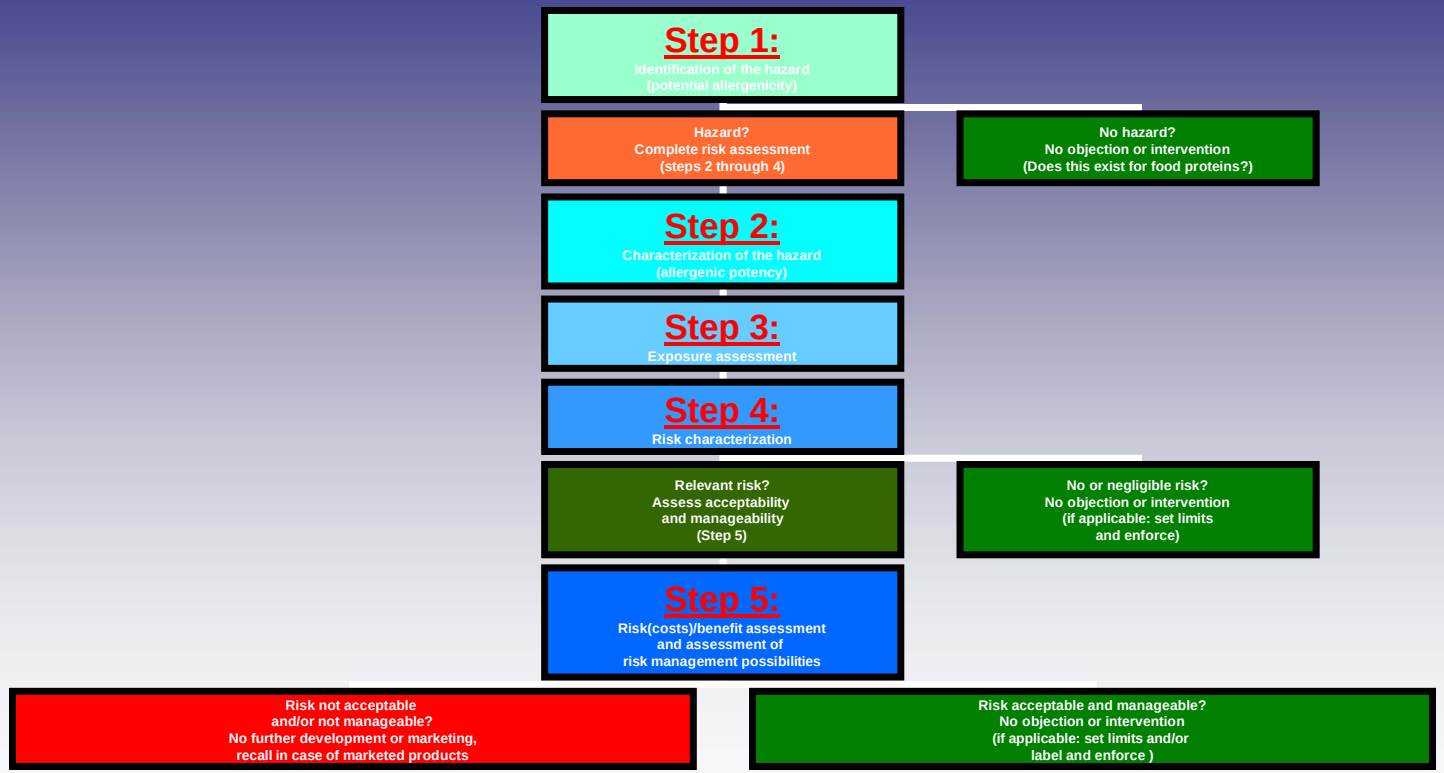
negative: Was the dose too low?

positive: Which dose is tolerable?

- **may lead to false negative results; false positive results are assumed to be negligible**
- **recommended for diagnosis except in cases with a history of an anaphylactic shock**

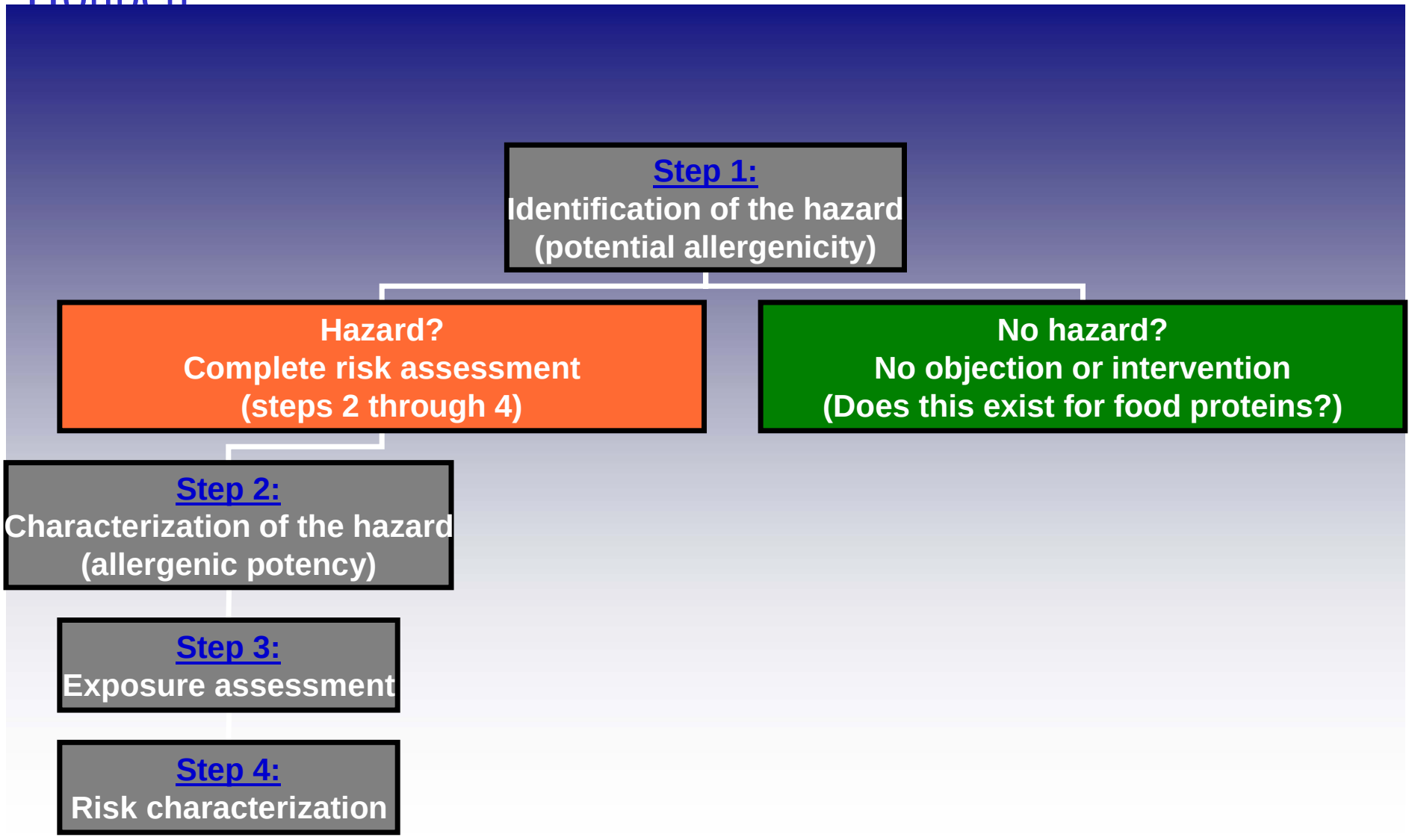
Risk analysis-based decision making for allergenic proteins; possible model

Source: T
NO Nutrition and Food Research NL,
Geert F Houben



Risk analysis-based decision making for allergenic proteins; possible model – risk assessment part

Source: TNO Nutrition and Food Research NL, Geert F Houben



Risk analysis-based decision making for allergenic proteins; possible model – risk management part

Source: TNO Nutrition and Food Research NL, Geert F Houben